

**DETECTION OF INHIBITORY SUBSTANCES IN MILK**

**DELVOTEST 5 PACK/Visual & DelvoScan Reader**

**For Raw and Finished Cow and Goat Milk**

**[Unless otherwise stated all tolerances are  $\pm 5\%$ ]**

**SAMPLES**

1. Laboratory Requirements (see CP, item 33 & 34), except \_\_\_\_\_
  - a. For Appendix N testing, see Appendix N General Requirements form, items 9 - 14 \_\_\_\_\_

**APPARATUS**

2. See Cultural Procedures, items 1-23, except \_\_\_\_\_
  - a. For Appendix N testing, see Appendix N General Requirements form, items 1-7 \_\_\_\_\_
3. Dry incubator and/or water bath  $64\pm 2^{\circ}\text{C}$  \_\_\_\_\_
4. Heating block and/or water bath thermostatically controlled at  $82\pm 2^{\circ}\text{C}$ , for confirmation \_\_\_\_\_
5. Fixed Volume or electronic 100  $\mu\text{L}$  pipettor (\_\_\_\_\_) with appropriate tips \_\_\_\_\_
6. Forceps, Tablet Dispenser, or equivalent \_\_\_\_\_
7. Test tubes, 10 mL or greater for beta-lactam confirmation (optional) \_\_\_\_\_
8. Timer \_\_\_\_\_
9. **DelvoScan Reader (optional) (approved for white milk only)** \_\_\_\_\_
  - a. Software version \_\_\_\_\_
  - b. Scanner (\_\_\_\_\_) \_\_\_\_\_
    1. Instrument calibrated once every 30 days \_\_\_\_\_
    2. Latest calibration date \_\_\_\_\_
  - c. Computer with Windows operating system \_\_\_\_\_
  - d. Printer \_\_\_\_\_
  - e. Kodak Q-60 5x7" color reference photo card \_\_\_\_\_

f. Black/Dark cloth for scanner background \_\_\_\_\_

### **MATERIALS**

10. See Cultural Procedures, items 24-32 \_\_\_\_\_

11. Delvotest P 5 Pack Kit \_\_\_\_\_

a. Kit: Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

b. Bottle of nutrient tablets Lot # \_\_\_\_\_

c. Store kits at 0-15C \_\_\_\_\_

d. Opened bottles of nutrient tablets at  
room temperature Date Opened: \_\_\_\_\_

e. Run a positive control (item 13) and negative  
control (item 14) with each new lot of kits, give  
appropriate reactions, records maintained \_\_\_\_\_

12. Beta-lactamase, 10,000,000 IU/mL (not required if beta-  
lactamase is not used for confirmation) \_\_\_\_\_

a. Stored as per manufacturer's instructions \_\_\_\_\_

b. Do not use beyond expiration date \_\_\_\_\_

1. Mfg. \_\_\_\_\_ Lot No. \_\_\_\_\_ Exp. Date \_\_\_\_\_

c. Test each lot for suitability, add beta-lactamase to  
5.0 ppb positive control (item 13) and add to one  
(1) well, beta-lactamase neutralizes positive control;  
records maintained \_\_\_\_\_

13. Commercial Standard, 5.0 ppb Penicillin G Positive Control \_\_\_\_\_

a. Store according to label instructions \_\_\_\_\_

Mfg. \_\_\_\_\_ Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

b. Rehydrate as per manufacturer's instructions \_\_\_\_\_

c. Test for suitability each time prepared, add to one (1)  
well, must produce appropriate reaction (purple) or  
DelvoScan positive (<POS>); records maintained \_\_\_\_\_

d. Store solution at 0-4.4C for no more than 2 days \_\_\_\_\_

- e. Or, distribute sufficient amount in small containers, seal and freeze at -15C or below in non-frost-free freezer (or in a small styrofoam box, placed in center of frost-free freezer) for no more than 2 months

Date prep. \_\_\_\_\_ Lab Exp. Date: \_\_\_\_\_

#### 14. Negative Control

1. Inhibitor Free Milk (fluid milk product with milkfat 0.00 to 3.5%, total solids < 13%)
  - a. Test for suitability each time prepared, add to one (1) well, must produce appropriate reaction (yellow) or DelvoScan negative (<NEG>); records maintained

### TECHNIQUE

#### 15. Test Procedure

- a. Identify samples
- b. Use one negative and one positive control with each set of samples run ( $\leq$  94 samples)
- c. Depending on the number of samples to be tested, take out a sufficient number of whole multiplates and/or cut off the number of wells needed
- d. Remove aluminum top foil and using forceps or tablet dispenser add one nutrient tablet to each test well
- e. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc or vortex, use within 3 minutes
- f. Pipetting procedure
  1. With tip securely fastened to the end of the pipettor and the pipettor in a vertical position, depress the plunger to the first stop or for electronic pipettors as per manufacturer
  2. With the plunger still depressed, insert tip 1 cm below surface of the sample (avoid foam)
  3. Release plunger **slowly** allowing tip to fill (quickly releasing the plunger will cause inaccurate filling and may foul pipettor)
  4. Remove tip from sample and depress plunger to empty tip back into sample

5. If blow out type pipettor used, press the plunger to the second stop to completely empty the tip \_\_\_\_\_
6. Press plunger to first stop and repeat 2 and 3 above \_\_\_\_\_
7. Touch off to a dry spot on the inside of the sample container \_\_\_\_\_
- g. Add 100 µL of mixed sample/control to a test well \_\_\_\_\_
- h. Change pipettor tips for each sample and control \_\_\_\_\_
- i. Close used wells carefully with sealing sheets, provided with test kit \_\_\_\_\_
- j. Place sealed multiplates and/or blocks in pre-warmed dry incubator or water bath and incubate at 64±2C for the time period specified by the manufacturer. Time is approximate and test is complete when controls give proper color reactions \_\_\_\_\_
- k. Remove from dry incubator or water bath and visually read test result from the bottom side, see item 17b for interpretation \_\_\_\_\_
- l. Optionally read multiplates with DelvoScan Reader (Multiplates from water bath must be dried off prior further handling) \_\_\_\_\_
  1. Start DelvoScan software by double click on icon \_\_\_\_\_
    - a. Select test (Delvo P 5 pack) and press next \_\_\_\_\_
    - b. Input operator, sample and test kit data \_\_\_\_\_
      1. Enter number of test on scan-bed \_\_\_\_\_
      2. Enter test kit lot number \_\_\_\_\_
      3. Enter sample data \_\_\_\_\_
    - c. Press 'SCAN' and results will be displayed \_\_\_\_\_
    - d. Press 'PRINT' to obtain hard copy result data \_\_\_\_\_
- m. Samples with purple color on all **or part** of solid medium, or that read as DelvoScan positive (< POS >) must be promptly confirmed \_\_\_\_\_
- n. Samples that are yellow after incubation or that read as DelvoScan negative (< NEG >), inhibitor not detected (**Not Found**) \_\_\_\_\_

16. Laboratory Procedure, Confirmation

a. Inhibitor confirmation and optional beta-lactamase confirmation

1. Prepare and label tubes with 5 mL of each suspect sample
2. Prepare and label a tube with 5 mL of inhibitor free milk (item 14)
3. Prepare and label a tube with 5 mL of positive control milk
4. Heat all tubes to  $82 \pm 2^\circ\text{C}$  for 2 minutes (TC required)
5. Remove and cool rapidly in an ice bath to room temperature
6. Use of beta-lactamase (**optional by State Regulatory Agency**)
  - a. Prepare and label two tubes with 5 mL of each suspect sample and two tubes for the positive and negative controls
  - b. Heat all tubes to  $82 \pm 2^\circ\text{C}$  for 2 minutes (TC required)
  - c. Remove and cool rapidly in an ice bath to room temperature
  - d. Add 200  $\mu\text{L}$  (2 x 100  $\mu\text{L}$ ) of beta-lactamase to one tube of each sample and control
  - e. Agitate, shake or vortex, to thoroughly mix tubes and let stand 15 minutes at room temperature
7. Cut off enough wells for all sample and control tubes
  - a. Or, alternatively Delvotest P ampoules may be used (must be certified for this procedure)
8. Remove top foil and add one (1) nutrient tablet to each test well
9. Vortex tubes and add 100  $\mu\text{L}$  of mixed sample/control to a test well (as per 15f1-7 above), identify samples, repeat for all samples and controls
10. Change pipettor tips for each sample and control

11. Close wells carefully with sealing strips, enclosed in test kit \_\_\_\_\_
12. Place sealed blocks in pre-warmed dry incubator or water bath and incubate at 64±2C for the time period specified by the manufacturer. Time is approximate and test is complete when controls give proper color reactions \_\_\_\_\_
13. Remove from dry incubator or water bath and visually read test result from the bottom side \_\_\_\_\_
14. Optionally read multiplates with DelvoScan Reader (Multiplates from water bath must be dried off prior further handling) \_\_\_\_\_
  - a. Start DelvoScan software by double click on icon \_\_\_\_\_
    1. Select test (Delvo P 5 pack) and press next \_\_\_\_\_
    2. Input operator, sample and test kit data \_\_\_\_\_
      - a. Enter number of test on scan-bed \_\_\_\_\_
      - b. Enter test kit lot number \_\_\_\_\_
      - c. Enter sample data \_\_\_\_\_
    3. Press 'SCAN' and results will be displayed \_\_\_\_\_
    4. Press 'PRINT' to obtain hard copy result data \_\_\_\_\_
15. Record the color reactions or DelvoScan test result of all samples and controls \_\_\_\_\_
16. Controls give appropriate reactions/colors, if not repeat test \_\_\_\_\_
- b. Interpretation of Confirmation Tests \_\_\_\_\_
  1. Wells that are yellow or yellow/purple or that read as DelvoScan negative (<NEG>) after incubation, inhibitor not detected (**Not Found**) \_\_\_\_\_
  2. Wells that are purple or that read as DelvoScan positive (<POS>) after incubation, inhibitor present (**Positive**) \_\_\_\_\_

3. Interpretation of optional beta-lactamase test: \_\_\_\_\_

- a. If the untreated milk sample is yellow or yellow/purple, or DelvoScan negative (<NEG>) **and** the corresponding beta-lactamase treated milk sample is yellow or yellow/purple, or DelvoScan negative (<NEG>), inhibitor not Detected (**Not Found**) \_\_\_\_\_
- b. If the untreated milk sample is purple or DelvoScan positive (<POS>) **and** the corresponding beta-lactamase treated milk sample is yellow or yellow/purple, or DelvoScan negative (<NEG>), sample is **Positive for beta-lactam** \_\_\_\_\_
- c. If the untreated milk sample is purple or DelvoScan positive (<POS>) **and** the corresponding beta-lactamase treated milk sample is also purple or DelvoScan positive (<POS>), sample is **Positive for inhibitor (non-beta-lactam), report to State regulatory agency** \_\_\_\_\_
- d. If the untreated milk sample is yellow or yellow/purple or DelvoScan negative (<NEG>) **and** the corresponding beta-lactamase treated milk sample is purple or DelvoScan positive (<POS>), test is invalid, repeat test \_\_\_\_\_

- c. **Confirmation of Appendix N samples**, see Appendix N General Requirements form item 12-13, perform confirmation as in items 15a-n above (**use of beta-Lactamase required**) and interpret as in item 16b3 above \_\_\_\_\_

17. Recording and Reporting (for Appendix N also see Appendix N General Requirements form, item 14) \_\_\_\_\_

- a. Record test performed, interpretation of unknowns (samples) and controls \_\_\_\_\_
- b. Report presence of inhibitor only for heated milk samples \_\_\_\_\_
- c. If inhibitor is not detected report as **Not Found** \_\_\_\_\_
- d. Report presence of inhibitor as **Positive (+)** or **Positive for beta-lactam** (if confirmed with beta-lactamase or item 16c) \_\_\_\_\_
- e. If inhibitor is present, plate counts cannot be reported \_\_\_\_\_